

WO9511974

Publication Title:

HUMAN PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR

Abstract:

Abstract of WO9511974

A human peroxisome proliferation activated receptor gene is purified from the environment in which it naturally occurs, and preferably provided within an expression vector.

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(54) Title: HUMAN PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR		
<pre> 10 20 30 40 50 60 70 80 90 100 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ATGTTGACAA GGAAGGCGC ACTCTGCCCC CTCTGCCCC TCGAGGCGCG GATCTAGAG AGCCCTTAT CTGAAGAGTT CTGCAAGAA ATGGGAAACA H V D T E S P L C P L S P L E A G D L E S P L S E E F L Q E H G N I TCCAAGAGAT TTGCAATCC ATGGGCGAGG ATAGTCTGG AAGCTTTGCC TTACGGAAT ACCAGTATTT AGGAAGCTGT CTTGGCTCAG ATGGCTCGST Q E I S Q S I G E D S S G S F G F T E Y Q Y L G S C P G S D G S V CATCAAGGAC AGGCTTTTCA CAGCTTGGAG CCGCTGCTCG GTGACTTATC CTGTGTGCG CGGCGGCTG GAGGAGTCTC CAGTGGAGC ATTGAACATC I T O T L S P A S S P S S V T Y P V V P G S V D E S P S G A L N I GAATGTAGAA TGTGCGGGA CAAGGCTCA GGTATCATY ACGGAGTCCA CGGTGTGAA GGTGCAAGG GCTTCTTGG GGAAGGATT CGACTCAAGC E C R I C G D K A S G Y H Y G V H A C E G C K G F F R R T I R L K L TGTGTATGA CAGTGGGAC CGGAGTGA AGATGAGAA AAGAAGAGA AACAAATGCC AGTATTGCG ATTTCACAG TGGCTTTCTG TGGGATGTC V Y D K C D R S C K I Q K K N R N K C Q Y C R F H K C L S V G M S ACACAAGCG ATTTGTTTG GAGCAATGCC AAGATCTGAG AAGCAAAAC TGAAGCAGA AATTCTTACC TGTGAACATG ACATAGAAGA TTCTGAACT H N A I R F G R H P R S E K A K L K A E I L T C E H D I E D S E T GCAGATCTCA AATCTTGCC CAAGAGAATC TAGGAGGCTT ACTTGAAGAA CTTCAACATG AACAGGTCA AAGCGCGGT CATCTCTCA GSAAGGCCA A D L X S L A K R I Y E A Y L K M F N H N K V K A R Y I L S G K A S GTAACAATCC AACTTTTGT ATACATGATA TGGAGACACT GTGTATGCT GAGAAGGCG TGTGCGCAA GCTGTGCGC AATGGCATCC AGAACAAGGA N H P P F V I H D H E T L C H A E X T L V A K L V A N G I Q N K E GGGGAGGTC CGCATCTTC ACTGCTGCA GTGCAAGTCA GTGAGAGCG TCAGGAGCT CAGGAAATC GCAAGGCA TCCAGGCTT CGCAACTTG A E V R I F H C C Q C T S V E T V T E L T E F A K A I P G F A N L GACCTGAAG ATCAAGTAC ATTCTAAAA TACGGAGTTT ATGAGGCAAT ATTCGCAATG CTGTCTTCTG TGTGAACAA AGACGGGATG CTGGTAGGCT D L H D Q V T L L K Y G V Y E A I F A H L S S V H N K D G M L V A Y ATGGAATGG GTTTATACT CGTGAATTC TAAAGGCT AAGGAAGCG TTCTGTGATA TCATGAAAC CAGTTTGTAT TTTGCCATGA AGTTCAATTC G N G F I T R E F L K S L R K P F C D I H E P K F D F A H X F N A ACTGGAAGT GATGACAGT ATATCTGCT TTTGTGCT GCTATCATY GCTGTGAGA TGTGCTGCG CTCTAAAGS TAGGACATY TGAAGAAATG L E L D O S D I S L F V A A I T C G D R P G L L N V G H I E K H CAGGAGGTA TTGTACATGT GCTCAGATC CAGCTGAGA GCAACGCGG GAGGATATC TTTCTCTTC CAAACTTCT TCAAAAATG GCAGAGCTCC Q E G I V H V L R L H L Q S N H P D D I F L F P K L L Q K H A O L R GCAAGCTGT GAGGAGCAT GCGAGCTGG TGCAGATAT CAAGAGAGG GAGTGGATG CTGCGTGCA CCGCTACTG CAGGAGATCT ACAGGAGAT Q L V T E H A Q L V O I I K K T E S O A A L H P L L Q E I Y R D H GTACTGA Y X </pre>		
(57) Abstract		
A human peroxisome proliferation activated receptor gene is purified from the environment in which it naturally occurs, and preferably provided within an expression vector.		

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DESCRIPTIONHuman Peroxisome Proliferator Activated ReceptorCross Reference to Related Application

This application is a continuation-in-part of Application Docket No. 202/041, titled "Human Peroxisome Proliferator Activated Receptor," filed October 22, 1993, by Mukherjee, the disclosure of which is incorporated herein by reference.

Field of the Invention

This invention relates to the cloning and uses of a human peroxisome proliferator activated receptor.

Background of the Invention

5 A peroxisome proliferator is an agent that induces peroxisomal proliferation. Peroxisome proliferators are a diverse group of chemicals which include unsaturated fatty acids, hypolipidemic drugs, herbicides, leukotriene antagonists, and plasticizers (for a review, see Green,
10 S., 43 Biochem. Pharmacol. 393-400, 1992). Hypolipidemic drugs such as clofibrates have been found to lower triglycerides and cholesterol levels in plasma and to be beneficial in the prevention of ischaemic heart disease in individuals with elevated levels of cholesterol (Havel,
15 R.J. and Kane, J.P., 13 Ann. Rev. Pharmac. 287-308, 1973). Therapeutic use of such drugs, however, is questioned because clofibrates are carcinogens in rats.

Peroxisome proliferator activated receptor (PPAR) is a member of the steroid receptor family. It is activated
20 by peroxisome proliferators. Issemann and Green, 347 Nature 645, 1990, cloned a mouse peroxisome proliferator activated receptor (mPPAR) gene from a mouse liver complementary DNA (cDNA) library. Göttlicher et al., 89 Proc. Nat. Acad. Sci. USA 4653-4657, 1992, cloned a rat
25 peroxisome proliferator activated receptor (rPPAR) gene from a rat liver cDNA library. PPARs from mouse and rat share 97% homology in amino acid sequence and a

particularly well-conserved putative ligand-binding domain. Three members of the *Xenopus* nuclear hormone receptor superfamily have also been found to be structurally and functionally related to the mPPAR
5 (Dreyer et al., 68 Cell 879-887, 1992).

Schmidt et al., 6 Molecular Endocrinology 1634-1641, 1992, cloned a steroid hormone receptor gene, NUC1, from a human osteosarcoma cell cDNA library. The homology between amino acid sequence of NUC1 and that of the mouse
10 PPAR is only 62%. Thus, although it is clear that NUC1 is a member of the PPAR receptor group, it remains to be determined whether NUC1 is the human homolog of the mouse PPAR or a new member of the PPAR family.

Sher et al., 32 Biochemistry 5598-5604, 1993, cloned
15 a human PPAR gene from a human liver cDNA library. This clone has 85% nucleotide sequence homology and 91% amino acid sequence homology with the mPPAR clone.

Summary of the Invention

The present invention relates to the cloning of a
20 human PPAR gene, hPPAR1. The protein encoded by hPPAR1 has 92% homology with the mouse PPAR. It is different from the human PPAR cloned by Sher et al., supra, at two locations in the amino acid sequence, i.e., amino acids 268 and 296.

25 The hPPAR1 clone can be used for the expression of large amounts of hPPAR1. This human PPAR clone is also useful for screening compounds for improved pharmacological profiles for the treatment of hyperlipidemia with higher potency, efficacy, and fewer
30 side effects. Specifically, the human PPAR clone can be used to screen for compounds active as primary endogenous inducers of the human PPAR. In addition, it is useful for establishing the tissue specific expression pattern of human PPAR. For example, a Northern blot can be used to
35 reveal tissue specific expression of the gene to aid treatment of diseases such as atherosclerosis.

Thus, in a first aspect, the invention features a purified nucleic acid encoding a human PPAR with the nucleotide base sequence shown in Figure 1, and given as SEQ ID NO. 1. By purified nucleic acid is meant that the
5 nucleic acid is separated from its natural environment and from other nucleic acids.

In a second aspect, the present invention features a vector containing the human PPAR gene. This vector may be used for multiplication of the human PPAR gene or
10 expression of the human PPAR gene.

In a preferred embodiment, the vector is an expression vector. In one example, the expression vector is used to make a recombinant human PPAR nucleic acid, which can be used as a specific probe for DNA or RNA
15 complementary to the human PPAR sequence. In another example, the expression vector is used to express human recombinant PPAR protein.

By vector is meant a plasmid or viral DNA molecule into which either a cDNA or a genomic DNA sequence is
20 inserted.

By expression vector is meant a vector that directs protein synthesis from a promoter. In a preferred embodiment, either vector pBacPAK8 (Clontech) or vector pBacPAK9 (Clontech) is used to express the human PPAR in
25 insect cells. In another preferred embodiment, vector pYES2 (Invitrogen) is used to express the human PPAR in yeast cells. In yet another preferred embodiment, pBKCMV (Stratagene) is used to express the human PPAR in mammalian cells.

30 By recombinant human PPAR is meant a non-naturally expressed human PPAR.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred EmbodimentsDrawings

Figure 1 is the nucleotide and amino acid sequence of hPPAR1; and

5 Figure 2 is a comparison of the amino acid sequences of hPPAR1 and the mouse PPAR.

What follows is an example of the cloning of a human PPAR. Those of ordinary skill in the art will recognize that equivalent procedures can be readily used to isolate
10 human PPAR from cDNA libraries or genomic libraries of other tissues than that exemplified below, namely the liver.

In general, the cloning of the human PPAR involved probing a human liver cell cDNA library with a labeled
15 EcoRI-BglII fragment (nucleotides 450-909) of the rat PPAR (459 bases). The sequence of the probe is shown in Götlicher et al. supra.

The recipes for buffers, mediums, and solutions in the following examples are given in J. Sambrook, E. F.
20 Fritsch, and T. Maniatis, Molecular Cloning: A Laboratory Manual, 2 Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

Example 1: Cloning of a human PPAR

A human PPAR subtype, hPPAR1, was cloned from a human
25 liver 5'-stretch cDNA library (Clontech #HL1115a) in lambda gt10 phages. C600-Hfl coli (Clontech) was grown overnight in LB broth supplemented with 0.2% maltose. A required amount of phage (corresponding to 2 million plaques) was mixed with 200 microliters of 10 mM MgCl₂/10
30 mM CaCl₂ and 1.5 milliliters of the overnight C600-Hfl coli and incubated at 37°C for 30 minutes. Soft LB agarose was added at 48°C, mixed and poured onto prewarmed 22x22 cm rectangular LB agar plates and incubated overnight at 37°C.

35 Plaque lifts were performed by chilling the plates at 4°C to harden the top agarose and prevent it from peeling,

marking a nylon or nitrocellulose filter on the surface contacting the plaques, laying the filter on the surface without trapped air bubbles, and leaving it for about a minute. A number of asymmetric dots were inserted with
5 Indian ink with a syringe and needle so that the ink soaked into the agar. The sheets were then peeled gently away, and laid plaque side up on two sheets of Whatman 3MM soaked in denaturing solution, and left for about 2 minutes. The sheets were then peeled away and immersed in
10 a standard neutralizing solution for 5 minutes, immersed in 5X SSC, air dried, and baked at 80°C under vacuum, for 2 hours.

The filters were prehybridized in 40% formamide, 5X SSC, 0.1 % SDS, 1X Denhardt, and 100 ng/ml denatured
15 salmon sperm DNA at 37°-42°C for 1 hour. Labeled DNA probe (1 million cpm/ml) was denatured by heating at 100°C for 10 minutes, chilled, and then added to the prehybridization solution, and hybridized at 37°-42°C overnight. The filters were washed in 2X SSC and, 0.1%
20 SDS at 42°C or higher temperature.

Positive plaques were identified and purified by rescreening two more times. The probe was labeled by nick-translation.

Phage stocks were made by isolating and removing a
25 well separated plaque with the narrow end of an autoclaved Pasteur pipette, immersing it in 1 ml of standard SM buffer, and adding a drop of chloroform. This was left for a few hours at room temperature (20°C-24°C) or overnight at 4°C, vortexed, and centrifuged.

30 The cDNA insert was amplified by polymerase chain reactions (PCR). 20 microliters of phage stock was used in 100 microliters of standard PCR reaction buffer, by adding all components except Polymerase. This mixture was heated to 99°C, and Vent DNA polymerase (Biolabs) was
35 added to start the PCR cycles. The PCR conditions were 95°C 1 minute, 72°C 1 minute, 72°C 3 minutes (1 minute per

kilobase) for 30 cycles, 72°C 5 minutes, and kept at 4°C till further utilized.

The applicant isolated a clone from the cDNA library using an EcoRI-BglII fragment (nucleotides 450-909) of the
5 rat PPAR (459 bases) as a probe and the hybridization conditions provided above. This clone was purified and its sequence defined. This sequence is shown in Figure 1, and as SEQ. ID. NO. 1. Figure 2 is a comparison of mPPAR and hPPAR1 amino acid sequences with those amino acids
10 having identity between the two sequences enclosed in blocks.

Example 2: Northern blot analysis

A human multiple tissue Northern blot was purchased from Clontech. Screening was done following the
15 manufacturer's protocol. The blot was prehybridized in 5X SSPE, 10X Denhardt's solution, 100µg/ml of freshly denatured salmon sperm DNA, 50% formamide and 2% SDS for 3 hours at 42°C. DNA from the EcoRI site at position 1025 of the coding region to the end of the cloned gene was
20 used as probe (see Figure 1). This DNA was labeled by random priming, boiled and added at a concentration of 1 million cpm/ml of prehybridization solution. Hybridization was carried out for 13 hours at 42°C. The blot was then washed in 2X SSC, 0.05% SDS at room
25 temperature followed by two washes in 0.1X SSC, 0.1% SDS at 50°C and exposed to X-ray film.

A specific band of about 10 kilobase was observed in all tissues except the brain. Maximal expression was observed in skeletal muscle, followed by heart, placenta,
30 pancreas, liver, kidney, and lung. The expression of hPPAR1 gene is therefore observed in tissues known to express PPARs in other species.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

5 (A) NAME: LIGAND PHARMACEUTICALS, INC.
(B) STREET: 9393 Towne Centre Drive
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: United States of America
(F) POSTAL CODE (ZIP): 92121
10 (G) TELEPHONE: (619) 535-3900
(H) TELEFAX: (619) 535-3906

(ii) TITLE OF INVENTION: HUMAN PEROXISOME
PROLIFERATOR
15 ACTIVATED RECEPTOR

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:

20 (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM compatible
(C) OPERATING SYSTEM: IBM P.C. DOS
(Version 5.0)
(D) SOFTWARE: WordPerfect (Version 5.1)

25 (v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: To Be Assigned

(vi) PRIOR APPLICATION DATA:

30 (A) APPLICATION NUMBER: 08/141,500
(B) FILING DATE: 22-OCT-1993

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/143,215
(B) FILING DATE: 26-OCT-1993

35

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 1407 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 1:

	ATG	GTG	GAC	ACG	GAA	AGC	CCA	CTC	TGC	CCC	CTC	TCC	CCA	39
	Met	Val	Asp	Thr	Glu	Ser	Pro	Leu	Cys	Pro	Leu	Ser	Pro	
					5					10				
5	CTC	GAG	GCC	GGC	GAT	CTA	GAG	AGC	CCG	TTA	TCT	GAA	GAG	78
	Leu	Glu	Ala	Gly	Asp	Leu	Glu	Ser	Pro	Leu	Ser	Glu	Glu	
		15					20					25		
	TTC	CTG	CAA	GAA	ATG	GGA	AAC	ATC	CAA	GAG	ATT	TCG	CAA	117
10	Phe	Leu	Gln	Glu	Met	Gly	Asn	Ile	Gln	Glu	Ile	Ser	Gln	
				30						35				
	TCC	ATC	GGC	GAG	GAT	AGT	TCT	GGA	AGC	TTT	GGC	TTT	ACG	156
	Ser	Ile	Gly	Glu	Asp	Ser	Ser	Gly	Ser	Phe	Gly	Phe	Thr	
		40				45					50			
	GAA	TAC	CAG	TAT	TTA	GGA	AGC	TGT	CCT	GGC	TCA	GAT	GGC	195
15	Glu	Tyr	Gln	Tyr	Leu	Gly	Ser	Cys	Pro	Gly	Ser	Asp	Gly	
			55					60					65	
	TCG	GTC	ATC	ACG	GAC	ACG	CTT	TCA	CCA	GCT	TCG	AGC	CCC	234
	Ser	Val	Ile	Thr	Asp	Thr	Leu	Ser	Pro	Ala	Ser	Ser	Pro	
					70					75				
20	TCC	TCG	GTG	ACT	TAT	CCT	GTG	GTC	CCC	GGC	AGC	GTG	GAC	273
	Ser	Ser	Val	Thr	Tyr	Pro	Val	Val	Pro	Gly	Ser	Val	Asp	
		80					85					90		
	GAG	TCT	CCC	AGT	GGA	GCA	TTG	AAC	ATC	GAA	TGT	AGA	ATC	312
25	Glu	Ser	Pro	Ser	Gly	Ala	Leu	Asn	Ile	Glu	Cys	Arg	Ile	
				95					100					
	TGC	GGG	GAC	AAG	GCC	TCA	GGC	TAT	CAT	TAC	GGA	GTC	CAC	351
	Cys	Gly	Asp	Lys	Ala	Ser	Gly	Tyr	His	Tyr	Gly	Val	His	
		105				110					115			
	GCG	TGT	GAA	GGC	TGC	AAG	GGC	TTC	TTT	CGG	CGA	ACG	ATT	390
30	Ala	Cys	Glu	Gly	Cys	Lys	Gly	Phe	Phe	Arg	Arg	Thr	Ile	
			120					125					130	
	CGA	CTC	AAG	CTG	GTG	TAT	GAC	AAG	TGC	GAC	CGC	AGC	TGC	429
	Arg	Leu	Lys	Leu	Val	Tyr	Asp	Lys	Cys	Asp	Arg	Ser	Cys	
				135						140				
35	AAG	ATC	CAG	AAA	AAG	AAC	AGT	TTC	AAA	TGC	CAG	TAT	TGT	468
	Lys	Ile	Gln	Lys	Lys	Asn	Arg	Asn	Lys	Cys	Gln	Tyr	Cys	
		145					150					155		
	CGA	TTT	CAC	AAG	TGC	CTT	TCT	GTC	GGG	ATG	TCA	CAC	AAC	507
40	Arg	Phe	His	Lys	Cys	Leu	Ser	Val	Gly	Met	Ser	His	Asn	
				160					165					

	GCG	ATT	CGT	TTT	GGA	CGA	ATG	CCA	AGA	TCT	GAG	AAA	GCA	546
	Ala	Ile	Arg	Phe	Gly	Arg	Met	Pro	Arg	Ser	Glu	Lys	Ala	
	170					175					180			
5	AAA	CTG	AAA	GCA	GAA	ATT	CTT	ACC	TGT	GAA	CAT	GAC	ATA	585
	Lys	Leu	Lys	Ala	Glu	Ile	Leu	Thr	Cys	Glu	His	Asp	Ile	
			185					190					195	
	GAA	GAT	TCT	GAA	ACT	GCA	GAT	CTC	AAA	TCT	CTG	GCC	AAG	624
	Glu	Asp	Ser	Glu	Thr	Ala	Asp	Leu	Lys	Ser	Leu	Ala	Lys	
					200					205				
10	AGA	ATC	TAC	GAG	GCC	TAC	TTG	AAG	AAC	TTC	AAC	ATG	AAC	663
	Arg	Ile	Tyr	Glu	Ala	Tyr	Leu	Lys	Asn	Phe	Asn	Met	Asn	
		210					215					220		
15	AAG	GTC	AAA	GCC	CGG	GTC	ATC	CTC	TCA	GGA	AAG	GCC	AGT	702
	Lys	Val	Lys	Ala	Arg	Val	Ile	Leu	Ser	Gly	Lys	Ala	Ser	
				225					230					
	AAC	AAT	CCA	CCT	TTT	GTC	ATA	CAT	GAT	ATG	GAG	ACA	CTG	741
	Asn	Asn	Pro	Pro	Phe	Val	Ile	His	Asp	Met	Glu	Thr	Leu	
	235					240					245			
20	TGT	ATG	GCT	GAG	AAG	ACG	CTG	GTG	GCC	AAG	CTG	GTG	GCC	780
	Cys	Met	Ala	Glu	Lys	Thr	Leu	Val	Ala	Lys	Leu	Val	Ala	
			250					255					260	
	AAT	GGC	ATC	CAG	AAC	AAG	GAG	GCG	GAG	GTC	CGC	ATC	TTT	819
	Asn	Gly	Ile	Gln	Asn	Lys	Glu	Ala	Glu	Val	Arg	Ile	Phe	
					265					270				
25	CAC	TCG	TGC	CAG	TGC	ACG	TCA	GTG	GTG	ACC	GTC	ACG	GAG	858
	His	Cys	Cys	Gln	Cys	Thr	Ser	Val	Glu	Thr	Val	Thr	Glu	
		275					280					285		
30	CTC	ACG	GAA	TTC	GCC	AAG	GCC	ATC	CCA	GGC	TTC	GCA	AAC	897
	Leu	Thr	Glu	Phe	Ala	Lys	Ala	Ile	Pro	Gly	Phe	Ala	Asn	
				290					295					
	TTG	GAC	CTG	AAC	GAT	CAA	GTG	ACA	TTG	CTA	AAA	TAC	GGA	936
	Leu	Asp	Leu	Asn	Asp	Gln	Val	Thr	Leu	Leu	Lys	Tyr	Gly	
	300					305					310			
35	GTT	TAT	GAG	GCC	ATA	TTC	GCC	ATG	CTG	TCT	TCT	GTG	ATG	975
	Val	Tyr	Glu	Ala	Ile	Phe	Ala	Met	Leu	Ser	Ser	Val	Met	
			315					320					325	
	AAC	AAA	GAC	GGG	ATG	CTG	GTA	GCG	TAT	GGA	AAT	GGG	TTT	1014
	Asn	Lys	Asp	Gly	Met	Leu	Val	Ala	Tyr	Gly	Asn	Gly	Phe	
					330					335				
40	ATA	ACT	CGT	GAA	TTC	CTA	AAA	AGC	CTA	AGG	AAA	CCG	TTC	1053
	Ile	Thr	Arg	Glu	Phe	Leu	Lys	Ser	Leu	Arg	Lys	Pro	Phe	
		340					345					350		

[illegible]

30 (i) SEQUENCE CHARACTERISTICS:

35 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 2

Leu Glu Ala Gly Asp Leu Glu Ser Pro Leu Ser Glu Glu
15 20 25

11

	Phe	Leu	Gln	Glu	Met	Gly	Asn	Ile	Gln	Glu	Ile	Ser	Gln
				30					35				
	Ser	Ile	Gly	Glu	Asp	Ser	Ser	Gly	Ser	Phe	Gly	Phe	Thr
	40					45					50		
5	Glu	Tyr	Gln	Tyr	Leu	Gly	Ser	Cys	Pro	Gly	Ser	Asp	Gly
			55					60					65
	Ser	Val	Ile	Thr	Asp	Thr	Leu	Ser	Pro	Ala	Ser	Ser	Pro
					70					75			
10	Ser	Ser	Val	Thr	Tyr	Pro	Val	Val	Pro	Gly	Ser	Val	Asp
		80					85					90	
	Glu	Ser	Pro	Ser	Gly	Ala	Leu	Asn	Ile	Glu	Cys	Arg	Ile
				95					100				
	Cys	Gly	Asp	Lys	Ala	Ser	Gly	Tyr	His	Tyr	Gly	Val	His
	105					110					115		
15	Ala	Cys	Glu	Gly	Cys	Lys	Gly	Phe	Phe	Arg	Arg	Thr	Ile
			120					125					130
	Arg	Leu	Lys	Leu	Val	Tyr	Asp	Lys	Cys	Asp	Arg	Ser	Cys
					135					140			
20	Lys	Ile	Gln	Lys	Lys	Asn	Arg	Asn	Lys	Cys	Gln	Tyr	Cys
		145					150					155	
	Arg	Phe	His	Lys	Cys	Leu	Ser	Val	Gly	Met	Ser	His	Asn
				160					165				
	Ala	Ile	Arg	Phe	Gly	Arg	Met	Pro	Arg	Ser	Glu	Lys	Ala
	170					175					180		
25	Lys	Leu	Lys	Ala	Glu	Ile	Leu	Thr	Cys	Glu	His	Asp	Ile
			185					190					195
	Glu	Asp	Ser	Glu	Thr	Ala	Asp	Leu	Lys	Ser	Leu	Ala	Lys
					200					205			
30	Arg	Ile	Tyr	Glu	Ala	Tyr	Leu	Lys	Asn	Phe	Asn	Met	Asn
		210					215					220	
	Lys	Val	Lys	Ala	Arg	Val	Ile	Leu	Ser	Gly	Lys	Ala	Ser
				225					230				
	Asn	Asn	Pro	Pro	Phe	Val	Ile	His	Asp	Met	Glu	Thr	Leu
	235					240					245		
35	Cys	Met	Ala	Glu	Lys	Thr	Leu	Val	Ala	Lys	Leu	Val	Ala
			250					255					260

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	Asn	Gly	Ile	Gln	Asn	Lys	Glu	Ala	Glu	Val	Arg	Ile	Phe
					265					270			
	His	Cys	Cys	Gln	Cys	Thr	Ser	Val	Glu	Thr	Val	Thr	Glu
		275					280					285	
5	Leu	Thr	Glu	Phe	Ala	Lys	Ala	Ile	Pro	Gly	Phe	Ala	Asn
				290					295				
	Leu	Asp	Leu	Asn	Asp	Gln	Val	Thr	Leu	Leu	Lys	Tyr	Gly
	300					305					310		
	Val	Tyr	Glu	Ala	Ile	Phe	Ala	Met	Leu	Ser	Ser	Val	Met
10			315					320					325
	Asn	Lys	Asp	Gly	Met	Leu	Val	Ala	Tyr	Gly	Asn	Gly	Phe
					330					335			
	Ile	Thr	Arg	Glu	Phe	Leu	Lys	Ser	Leu	Arg	Lys	Pro	Phe
		340					345					350	
15	Cys	Asp	Ile	Met	Glu	Pro	Lys	Phe	Asp	Phe	Ala	Met	Lys
				355					360				
	Phe	Asn	Ala	Leu	Glu	Leu	Asp	Asp	Ser	Asp	Ile	Ser	Leu
	365					370					375		
	Phe	Val	Ala	Ala	Ile	Ile	Cys	Cys	Gly	Asp	Arg	Pro	Gly
20			380					385					390
	Leu	Leu	Asn	Val	Gly	His	Ile	Glu	Lys	Met	Gln	Glu	Gly
					395					400			
	Ile	Val	His	Val	Leu	Arg	Leu	His	Leu	Gln	Ser	Asn	His
		405					410					415	
25	Pro	Asp	Asp	Ile	Phe	Leu	Phe	Pro	Lys	Leu	Leu	Gln	Lys
				420					425				
	Met	Ala	Asp	Leu	Arg	Gln	Leu	Val	Thr	Glu	His	Ala	Gln
	430					435					440		
	Leu	Val	Gln	Ile	Ile	Lys	Lys	Thr	Glu	Ser	Asp	Ala	Ala
30			445					450					455
	Leu	His	Pro	Leu	Leu	Gln	Glu	Ile	Tyr	Arg	Asp	Met	Tyr
					460					465			468

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 468 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 3:

Met Val Asp Thr Glu Ser Pro Ile Cys Pro Leu Ser Pro
 5 10

10 Leu Glu Ala Asp Asp Leu Glu Ser Pro Leu Ser Glu Glu
 15 20 25

Phe Leu Gln Glu Met Gly Asn Ile Gln Glu Ile Ser Gln
 30 35

Ser Ile Gly Glu Glu Ser Ser Gly Ser Phe Gly Phe Ala
 40 45 50

15 Asp Tyr Gln Tyr Leu Gly Ser Cys Pro Gly Ser Glu Gly
 55 60 65

Ser Val Ile Thr Asp Thr Leu Ser Pro Arg Ser Ser Pro
 70 75

20 Ser Ser Val Ser Cys Pro Val Ile Pro Ala Ser Thr Asp
 80 85 90

Glu Ser Pro Gly Ser Ala Leu Asn Ile Glu Cys Arg Ile
 95 100

Cys Gly Asp Lys Ala Ser Gly Tyr His Tyr Gly Val His
 105 110 115

25 Ala Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Ile
 120 125 130

Arg Leu Lys Leu Val Tyr Asp Lys Cys Asp Arg Ser Cys
 135 140

30 Lys Ile Gln Lys Lys Asn Arg Asn Lys Cys Gln Tyr Cys
 145 150 155

Arg Phe His Lys Cys Leu Ser Val Gly Met Ser His Asn
 160 165

35 Ala Ile Arg Phe Gly Arg Met Pro Arg Ser Glu Lys Ala
 170 175 180

Lys Leu Lys Ala Glu Ile Leu Thr Cys Glu His Asp Leu
 185 190 195

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	Lys	Asp	Ser	Glu	Thr	Ala	Asp	Leu	Lys	Ser	Leu	Gly	Lys
					200					205			
	Arg	Ile	His	Glu	Ala	Tyr	Leu	Lys	Asn	Phe	Asn	Met	Asn
	210						215					220	
5	Lys	Val	Lys	Ala	Arg	Val	Ile	Leu	Ala	Gly	Lys	Thr	Ser
				225					230				
	Asn	Asn	Pro	Pro	Phe	Val	Ile	His	Asp	Met	Glu	Thr	Leu
	235					240					245		
10	Cys	Met	Ala	Glu	Lys	Thr	Leu	Val	Ala	Lys	Met	Val	Ala
			250					255					260
	Asn	Gly	Val	Glu	Asp	Lys	Glu	Ala	Glu	Val	Arg	Phe	Phe
					265					270			
	His	Cys	Cys	Gln	Cys	Met	Ser	Val	Glu	Thr	Val	Thr	Glu
		275					280					285	
15	Leu	Thr	Glu	Phe	Ala	Lys	Ala	Ile	Pro	Gly	Phe	Ala	Asn
				290					295				
	Leu	Asp	Leu	Asn	Asp	Gln	Val	Thr	Leu	Leu	Lys	Tyr	Gly
	300					305					310		
20	Val	Tyr	Glu	Ala	Ile	Phe	Thr	Met	Leu	Ser	Ser	Leu	Met
			315					320					325
	Asn	Lys	Asp	Gly	Met	Leu	Ile	Ala	Tyr	Gly	Asn	Gly	Phe
					330					335			
	Ile	Thr	Arg	Glu	Phe	Leu	Lys	Asn	Leu	Arg	Lys	Pro	Phe
		340					345					350	
25	Cys	Asp	Ile	Met	Glu	Pro	Lys	Phe	Asp	Phe	Ala	Met	Lys
				355					360				
	Phe	Asn	Ala	Leu	Glu	Leu	Asp	Asp	Ser	Asp	Ile	Ser	Leu
	365					370					375		
30	Phe	Val	Ala	Ala	Ile	Ile	Cys	Cys	Gly	Asp	Arg	Pro	Gly
			380					385					390
	Leu	Leu	Asn	Ile	Gly	Tyr	Ile	Glu	Lys	Leu	Gln	Glu	Gly
					395					400			
	Ile	Val	His	Val	Leu	Lys	Leu	His	Leu	Gln	Ser	Asn	His
		405					410					415	
35	Pro	Asp	Asp	Thr	Phe	Leu	Phe	Pro	Lys	Leu	Leu	Gln	Lys
				420					425				

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Met Val Asp Leu Arg Gln Leu Val Thr Glu His Ala Gln
430 435 440

Leu Val Gln Val Ile Lys Lys Thr Glu Ser Asp Ala Ala
445 450 455

5 Leu His Pro Leu Leu Gln Glu Ile Tyr Arg Asp Met Tyr
460 465 468

What is claimed is:

1. Purified nucleic acid comprising the nucleotide sequence shown in SEQ ID NO. 1.
2. A vector comprising said nucleic acid of claim
5 1.
3. Recombinant PPAR expressed from said nucleic acid of claim 1.

10 20 30 40 50 60 70 80 90 100
1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
ATGGTGGACA CGGAAAGCCC ACTCTGCCCC CTCCTCCACC TCGAGGCCGG CGATCTAGAG AGCCCGTTAT CTGAAGAGTT CTGCAAGAA ATGGGAAACA
M V D T E S P L C P L S P L E A G D L E S P L S E E F L Q E M G N I
200
TCCAAGAGAT TTCGCAATCC ATCGGCGAGG ATAGTTCIGG AAGCTTTGGC TTTACGGAAT ACCAGTATTT AGGAAGCTGT CCTGGCTCAG ATGGCTCGGT
Q E I S Q S I G E D S S G S F G F T E Y Q Y L G S C P G S D G S V
300
CATCAGGAC ACGCTTTCAC CAGCTTCGAG CCCCTCCICG GTGACTTATC CTGTGGTCCC CGGCAGCGTG GACGAGTCTC CCAGTGGAGC ATTGAACATC
I T D T L S P A S S P S S V T Y P V V P G S V D E S P S G A L N I
400
GAATGTAGAA TCTGCGGGGA CAAGGCCICA GGCTATCATT ACGGAGTCCA CGCGTGTGAA GGCTGCAAGG GCTTCTTTCG GCGAAGCATT CGACICAAGC
E C R I C G D K A S G Y H Y G V H A C E G C K G F F R R T I R L K L
500
TGGTGTATGA CAAGTGGGAC CGCAGCTGCA AGATCCAGAA AAAGAACAGA AACAAATGCC AGTATTGTCG ATTTCACAAG TGCCTTTCIG TCGGGATGTC
V Y D K C D R S C K I Q K K N R N K C Q Y C R F H K C L S V G M S
600
ACACAACGCG ATTGCTTTTG GACGAATGCC AAGATCTGAG AAGCAAAAC TGAAGCAGA AATTCTTACC TGTGAACATG ACATAGAAGA TTCTGAAACT
H N A I R F G R M P R S E K A K L K A E I L T C E H D I E D S E T
700
GCAGATCTCA AATCTCTGGC CAAGAGAATC TACGAGGCT ACTTGAAGAA CTTCAACATG AACAAAGTCA AAGCCCGGGT CATCTCTCA GGAAGGCCA
A D L K S L A K R I Y E A Y L K N F N M N K V K A R V I L S G K A S
800
GTAACAATCC ACCTTTTGTG ATACATGATA TGGAGACACT GTGTATGGCT GTGAGAGCGC GAGAAGACGC TGGTGGCCAA GCTGGTGGCC AATGGCATCC AGAACAAGGA
N N P P F V I H D M E T L C M A E K T L V A K L V A N G I Q N K E
900
GGCGGAGGTC CGCATCTTTC ACTGCTGCCA GTGCAGCTCA GTGGAGACCG TCACGGAGCT CACGGAATTC GCCAAGGCCA TCCCAGGCTT CGCAAACCTTG
A E V R I F H C C Q C T S V E T V T E L T E F A K A I P G F A N L
1000
GACCTGAACG ATCAAGTGAC ATTGCTAAAA TACGGAGTTT ATGAGGCCAT ATTGCCATG CTGCTTCTG TGATGAACAA AGACGGGATG CTGGTAGCGT
D L N D Q V T L L K Y G V Y E A I F A M L S S V M N K D G M L V A Y
1100
ATGGAATGG GTTTATAACT CGTGAATTCC TAAAAGCCT AAGGAAACCG TTCTGTGATA TCATGGAACC CAAGTTTGAT TTTGCCATGA AGTTCAATGC
G N G F I T R E F L K S L R K P F C D I M E P K F D F A M K F N A
1200
ACTGGAATG GATGACAGTG ATATCTCCCT TTTTGGGCT GCTATCATT GCTGTGGAGA TCGTCCIGGC CTTCTAAACG TAGGACACAT TGAATAAATG
L E L D D S D I S L F V A A I I C C G D R P G L L N V G H I E K M
1300
CAGGAGGTA TTGTACATGT GCTCAGACTC CACCTGCAGA GCAACCACCC GGACGATATC TTTCTCTCC CAAAACCTCT TCAAAAAATG GCAGACCTCC
Q E G I V H V L R L H L Q S N H P D D I F L F P K L L Q K M A D L R
1400
GGCAGCTGGT GACGGAGCAT GCGCAGCTGG TGCAGATCAT CAAGAAGACG GAGTGGGATG CTGCGCTGCA CCCGCTACTG CAGGAGATCT ACAGGGACAT
Q L V T E H A Q L V Q I I K K T E S D A A L H P L L Q E I Y R D M
1407
GTACTGA
Y X

FIG. 1

MVDTESPICP	LSPLEADLE	SPLSEEFLOE	MGNIQEISQS	IGESGSGFG	FADYQYLGC	PGSEGSVITD	TLSPASSPSS	VSCPVIIPAST	DESPGSGALNI	100
MVDTESPLCP	LSPLEAGDLE	SPLSEEFLOE	MGNIQEISQS	IGESGSGFG	FTEYQYLGC	PGSDGGSVITD	TLSPASSPSS	VITYPVMPPGSSV	DESPGSGALNI	100
ECRICGDKAS	GYHYGVHACE	GCKGFFRRTI	RKKLVYDKCD	RSCKIQKKNR	NKCQYCRFHK	CLSVGMSHNA	IRFGRMPRSE	KAKLKAEILT	CEHDLKDSET	200
ECRICGDKAS	GYHYGVHACE	GCKGFFRRTI	RKKLVYDKCD	RSCKIQKKNR	NKCQYCRFHK	CLSVGMSHNA	IRFGRMPRSE	KAKLKAEILT	CEHDIEDSET	200
ADLKSLSGKRI	YEAYLKNFNM	NKVKARVILDA	GKISNNPPFV	IHDMETLCMA	EKTLVAKMVA	NGVEDKEAEV	RHFHCCQQQMS	VETVTELTEF	AKAIPGFANL	300
ADLKSLSAKRI	YEAYLKNFNM	NKVKARVILS	GKASNNPPFV	IHDMETLCMA	EKTLVAKLVA	NGIQNKEAEV	RHFHCCQQQIS	VETVTELTEF	AKAIPGFANL	300
DLNDQVTLLK	YGVYEAIIFIM	LSSLMNKDGM	LIAYGNGFIT	REFLKNLRKP	FCDIMEPKFD	FAMKFNALEL	DDSDISLFLVA	AIICCGDRPG	LLNIGMIEUK	400
DLNDQVTLLK	YGVYEAIIFAM	LSSLMNKDGM	LVAYGNGFIT	REFLKSRLRP	FCDIMEPKFD	FAMKFNALEL	DDSDISLFLVA	AIICCGDRPG	LLNMGHIELM	400
QEGIVHVLKL	HLQSNHPDDIT	FLFPKLLQKM	VDLRQLVTEH	AQLVQMIIKKT	ESDAALHPLL	QEIYRDMY-				468
QEGIVHVLRL	HLQSNHPDDI	FLFPKLLQKM	ADLRQLVTEH	AQLVQIHKKT	ESDAALHPLL	QEIYRDMYX				469

FIG. 2